

嚼食檳榔對口腔黏膜病毒感染率之研究

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摘要

越來越多的資料顯示，病毒感染與多種口腔疾病有關，在疾病的發生、發展中扮演重要作用，病毒不僅可以直接導致口腔疾病，還可能通過感染引起全身系統性疾病，口腔逐漸成為許多病毒性疾病傳播的一個重要途徑，因此對可疑的口腔病例進行相關病毒學檢測具有臨床診斷與治療的重要意義。根據流行病學研究指出，口腔癌、口腔癌前病變（口腔白斑症、紅斑症和紅白斑症等）及口腔黏膜下纖維化症（oral submucous fibrosis, OSF）的發生與嚼食檳榔有密切關係。本研究選取20位正常人及25位口腔癌前病變或口腔黏膜下纖維化病例，利用聚合鏈鎖反應（PCR）偵測HPV-16病毒及HPV-18病毒對口腔黏膜之感染率。研究結果顯示，正常人樣本呈HPV-16陽性反應為5%，而HPV-18也同樣為5%，HPV-16及HPV-18雙重感染的比率為0%。實驗組的感染率以有、無嚼食檳榔習慣來區分，在HPV-16感染方面，有嚼食檳榔習慣者的感染率為31.6%，無嚼食檳榔習慣者的感染率為16.7%，全部實驗組樣本的感染率為28%，有嚼食檳榔習慣者與正常者比較結果在統計學上有顯著差異（ $p=0.035 < 0.05$ ）。在HPV-18感染方面，有嚼食檳榔習慣者的感染率為21%，無嚼食檳榔習慣者的感染率為0%，全部實驗組樣本的感染率為16%。不論有無嚼食檳榔之習慣與正常者比較其結果在統計學上皆無顯著性差異。在HPV-16及HPV-18雙重感染方面，有嚼食檳榔習慣者的感染率為15.8%，無嚼食檳榔習慣者的感染率為0%，全部樣本的感染率為12%，不論有無嚼食檳榔之習慣與正常者比較其結果在統計學上皆無明顯差異。因此HPV-16的感染對於嚼食檳榔者之口腔黏膜的纖維化或癌化可能具有某些作用，值得我們再做更進一步的研究。

關鍵詞：口腔癌前病變；口腔黏膜下纖維化；人類乳突瘤病毒；聚合鏈鎖反應

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參考文獻

- 1、許銘能（1998）口腔白斑及口腔癌自然病史之探討 國立台灣大學公共衛生研究所碩士論文
- 2、陳金勝與陳中和（1995）口腔鱗狀上皮細胞癌之統計分析報告 高雄醫學科學雜誌, 11, pp.582-588
- 3、蔡崇弘與邱清華（1990）台灣地區口腔癌發生率研究 中華牙誌, 9, pp.104-115
- 4、行政院衛生署（1995）醫院癌症登記申報手冊 保健處 台北
- 5、行政院衛生署（1996）醫院癌症登記申報手冊 保健處 台北
- 6、行政院衛生署（2001）中華民國九十年衛生統計 台北
- 7、國家衛生研究院癌症研究組（1998）口腔癌之治療共識 台灣癌症臨床研究合作組織
- 8、Alcalde RE, Terakado N, Otsuki K, and Matsumura T. (1997) Angiogenesis and expression of platelet-derived endothelial cell growth factor in oral squamous cell carcinoma. *Oncol* 54, pp.324-328.
- 9、Ashby J, Boyland E, and Styles JA. (1979) Betel nuts, arecaidine and oral cancer. *Lancet* 1, pp.112.
- 10、Bandara LR, Adamczewski JP, Hunt T, and La Thangue NB. (1991) Cyclin A and the retinoblastoma gene product complex with common transcription factor. *Nature* 352, pp.249-251
- 11、Barnard P and McMillan NA. (1999) The human papillomavirus E7 oncoprotein abrogates signaling mediated by interferon-alpha. *Virology* 259, pp.305-313.
- 12、Bates S, Phillips AC, Clark PA, Stott F, Peters G, Ludwig RL, and Vousden KH. (1998) p14ARF links the tumour suppressors RB and p53. *Nature* 395, pp.124-125.
- 13、Bernard HU, Chan SY, Manos MM, Ong CK, Villa LL, Delius H, Peyton CL, Bauer HM, and Wheeler CM. (1994) Identification and assessment of known and novel human papillomaviruses by polymerase chain reaction amplification, restriction fragment length poly-morphisms, nucleotide sequence, and phylogenetic algorithms. *J Infect Dis* 170, pp.1077-1085.
- 14、Bouquot JE, Weiland LH, and Kurland LT. (1988) Leukoplakia and carcinoma in situ synchronously associated with invasive oral/oropharyngeal carcinoma in Rochester, Minn. 1935-1984 *Oral Surg Oral Med Oral Path* 65, pp.199-207
- 15、Bouvard V, Storey A, Pim D, and Banks L. (1994) Characterization of the human papillomavirus E2 protein: evidence of trans-activation and trans-repression in cervical keratinocyte. *EMBO* 13, pp.5451-5459.
- 16、Brown DR, McClowry TL, Woods K, and Fife KH. (1999) Nucleotide sequence and characterization of human papillomavirus type 83, a novel genital papillomavirus. *Virology* 260, pp.165-172.
- 17、Chang KM. (1964) Betel nut chewing and mouth cancer in Taiwan. First report: Survey of disposition of mouth cancer in Taiwan. *Formosan Med Assoc* 63:437-448.
- 18、Cox MF, Scully C, and Maitland N. (1991) Viruses in the aetiology of oral carcinoma? Examination of the evidence. *British J of Oral and Maxillofacial Surg* 29, pp.381-387.
- 19、D'Costa J, Saranath D, Sanghvi V, and Mehta AR. (1998) Epstein-Barr virus in tobacco-induced oral cancers and oral lesions in patients from India. *J Oral Pathol Med* 27, pp.78-82
- 20、Doorbar J, Ely S, Sterling J, McLean C, and Crawford L. (1991) Specific interaction between HPV-16E1-E4 and cytokeratins results in collapse of the epithelial cell intermediate filament network. *Nature* 352, pp.824-827.
- 21、Drut RM, Day S, Drut R, and Mesner L. (1994) Demonstration of Epstein-Barr viral DNA in paraffin-embedded tissues of Burkitt's lymphoma from Argentina using the polymerase chain reaction and in situ hybridization. *Pediatr. Pathol.* 14, pp.101-109.
- 22、Dyson N, Howley PM, Munger K, and Harlow E. (1989) The human papillomavirus-16 E7 oncoprotein is able to bind to the retinoblastoma gene product. *Science* 243, pp.934-937.
- 23、Franceschi S, Munoz N, Bosch XF, Snijders PJ, and Wallboomers JM. (1996) Human papillomavirus and cancers of the upper aerodigestive tract: a review of epidemiological and experimental evidence. *Cancer Epidemiol Biomark Prevent* 5, pp.567-575.
- 24、Funk JO, Wanga S, Harry JB, Wspling E, Stillman B, and Galloway DA (1997) Inhibition of CDK activity and PCNA-dependent DNA replication by P21 is blocked by interaction with the HPV 16E7 oncoprotein. *Genes Dev* 11, pp.2090-2100.
- 25、Galloway DA. (1992) Serological assays for the detection of HPV antibodies. *ARC Sci Publ*, 119:147-161.
- 26、Hart KW, Williams OM, Thelwell N, Finder AN, Brown T, Borysiewicz LK, and Gelder CM. (2001) Novel method for detection, typing, and quantification of human papillomavirus in clinical sample. *J Clin Microbiol* 39, pp.3204-3212.
- 27、Horak ER, Leek R, Klenk N, Lejeune S, Smith K, Stuart N, Greenall M, Stepniewska K, and Harris AL. (1992) Angiogenesis, assessed by platelet/endothelial cell adhesion molecule antibodies, as indicator of node metastases and survival breast cancer. *Lancet* 340, pp.1120-4.
- 28、Honda R, Tanaka H, and Yasuda H. (1997) Oncoprotein MDM2 is a ubiquitin ligase E3 for tumor suppressor P53. *FEBS Lett* 420, pp.25-27.
- 29、Hwang SG, Lee D, Kim J, Seo T, and Choe J. (2002) Human papillomavirus type 16E7 binds to E2F1 and activates E2F1-driven transcription in a retinoblastoma protein-independent manner. *J Biol Chem* 277, pp.2923-2930.
- 30、Jewers RJ, Hildebrandt P, Ludlflow JW, Kell B, and McCane DJ. (1992) Regions of human papillomavirus type 16E7 oncoprotein required for immortalization of human keratinocyte. *J Virol* 66, pp.1329-1335.
- 31、Klingelutz AJ, Foster SA, and McDougall JK. (1996) Telomerase activation by the E6 gene product of human papillomavirus type 16. *Nature* 380, pp.79-82.
- 32、Kramer IR, Lucas RB, Pindborg JJ, and Sobin LH. (1978) Definition of leukoplakia and related lesions: an aid to studies on oral precancer. *Oral SURG Oral Med Oral Pathol* 46, pp.518-539.
- 33、Lenner P, Dillner J, and Wiklund F. (1995) Serum antibody responses against human papillomavirus in relation to tumor characteristic, response to treatment, and survival in carcinoma of the uterine cervix. *Cancer Immunol Immunother* 40, pp.201-205.
- 34、Liaw KL, Hsing AW, Chen CJ, Schiffman MH, Zhang TY, Hsieh CY, Greer CE, You SL, Huang TW, and Wu TC. (1995) Human papillomavirus and cervical neoplasia: a case-control study in Taiwan. *Int J Cancer*. 62, pp.565-571.
- 35、MacKonalk D G and Path F.R.C. (1987) Effects of arecaidine application to hamster cheek pouch. *J Oral Med* 42, pp.61-62.
- 36、Mendelsohn John (1995) The molecular basis of

cancer. Philadelphia:W.B.Saunders, pp.574. 37、 Miller EC, Swanson AB, Phillips DH, Fletcher TL, Liem A, and Miller JA. (1983) Structure-activity studies of the carcinogenesis in the mouse and rat of some naturally occurring and synthetic alkenylbenzene related to safrole and estragole. *Cancer Res* 43, pp.1124-34. 38、 Nelson B.S. and Heischober B. (1999) Betel nut: a common drug used by naturalized citizens from India, Far East Asia and the South Pacific Islands, *Ann Emerg Med* 34, pp.238-243. 39、 Ostrow RS, Manias DA, Clark BA, Okagaki T, Twigg LB, and Faras AJ. (1987) Detection of human papillomavirus DNA in invasive carcinomas of the cervix by in situ hybridization. *Cancer Res* 47, pp.649-53. 40、 Palevsky JM, Winkler B, Rabannus JP, Clark C, Chan S, Nizet V, and Scholnik GK.(1991) Characterization of in vivo expression of the human papillomavirus type 16 E4 protein in cervical biopsy tissues.*J Clon Invest* 87, pp.2132-2141. 41、 Paymaster JC. (1956) Cancer of the buccal mucosa:Clinical study of 650 cases in Indian patients. *Cancer* 9, pp.431-435. 42、 Pillai R, Balaram P, and Reddiar KS. (1992) Pathogenesis of oral submucous fibrosis.Relationship to risk factors associated with oral cancer. *Cancer* 69, pp.2011-2020. 43、 Pindborg J (1980) Oral cancer and precancer. Bristol, England :Wright. 44、 Piccini A, Storey A, Massimi P, and Banks L.(1995) Mutations in the human papillomavirus type 16 E2 protein identify multiple regions of the protein involved in binding to E1.*J Gen Virol* 76, pp.2909-2913. 45、 Saiki RK, Scharf S, Faloona F, Mullis KB, Horn GT, Erlich HA, and Arnheim N. (1985) Enzymatic amplification of β -globin genomic sequences and restriction site analysis diagnosis of sickle cell anemia. *Science* 230, pp.1350-1354. 46、 Schiffman MH and Brinton LA (1995) The epidemiology of cervical carcinogenesis.*Cancer,Suppl* 76:1888-1901. 47、 Schwarz E, Fresse UK, Gissmann L, Mayer W, Roggenbuck B,Stremlau A, and Zur Hausen H. (1985) Structure and transcription of human papillomavirus sequences in cervical carcinoma cells. *Nature* 314, pp.111-114. 48、 Silverman S. Jr, Gorsky M, and Lozada F. (1984) Oral leukoplakia and malignant transformation. A follow-up study of 257 patients *Cancer* 53, pp.563-8. 49、 Snijders PJ, Scholes AG, Hart CA, Jones AS, Vaughan ED, Woolgar JA, Meijer CJ, Walboomers JM, and Field JK. (1996) Prevalence of muscosotropic human papillomavirus in squamous-cell carcinomas of the head and neck. *Int J Cancer* 66, pp.464-469. 50、 Syrjanen SM (1999) New concepts on the role of human papillomavirus in cell cycle regulation. *Ann Med* 31, pp.175-187. 51、 Thiers V, Nakahima E, Kremsdorf D, Mack D, Schellekens H, Driss F, Goudeau A, Wands J, Sninsky J, and Tiollais P. (1988) Transmission of hepatitis B from hepatitis -B-seronegative subjects. *Lancet* 2, pp.1273-6. 52、 Thomas M and Banks L. (1999) Human papillomavirus (HPV) E6 internations with Bak are conserved amongst E6 protein from high and low risk HPV types. *J Gen Virol* 80, pp.1513-1517. 53、 Thorland EC, Myers SL, Persing DH, Sarkar G, McGovern RM, Gostout BS, and Smith DI. (2000) Human papillomavirus type 16 integrations in cervical tumors frequently occur in common fragile sites.*Cancer Res* 60, pp.5916-5921. 54、 Toki T, Kurman RJ, Park JS, Kessis T, Daniel RW, and Shah KV. (1991) Probable nonpapillomavirus etiology squamous cell carcinoma of the vulva in older woman: a clinicopathologic study using in situ hybridization and polymerase chain reaction. *Int J Gynecol Pathol* 10, pp.107-125. 55、 Van Ranst MA, Tachezy R, Delius H, and Burk RD. (1993) Taxonomy of the human papillomavirus Papillomavirus Rep 4, pp.61-65. 56、 Woodworth CD, Doniger J, and Dipaolo JA. (1989) Immortalisation of human foreskin keratinocytes by various haman papillomavirus DNAs corresponds to their association with cervical carcinoma. *J Virol* 63, pp.159-169. 57、 Zeffass-Thome K, Zwerschke W, Mannhardt B, Tindle R, Botz JW, and Jansen-Durr P.(1996) Inactivation of the cdk inhibitor p27KIP1 by the human papillomavirus type 16 E7 oncoprotein. *Oncogene* 13, pp.2323-2330. 58、 Zhou JA, Sun XY, Stenzel DJ, and Fraxer IH.(1991) Expression of vaccinia recombinant HPV 16L1 and L2 ORF proteins in epithelial cells is sufficient for assembly of HPV virion-like particles. *Virology* 181, pp.203-210. 59、 Zielinske GD, Snijders PJ, Rozendaal L, Voorhorst FJ, van der Linden HC, Runsink AP, de Schipper FA, and Meijer CJ. (2001) HPV presence precedes abnormal cytology in women developing cervical cancer and signals false negative smears. *Br J Cancer* 85, pp.398-404. 60、 Zur Huasen H. (2000) papillomavirus causing cancer:evasion from host-cell control in early events in carcinogenesis. *J Nat. cancer Int.* 92, pp.690-698.